Remarks

Claims 1-37 were the subject of the written restriction requirement dated February 17, 2006. Claims 1, 2, 26, and 27 are presently amended; all of the other claims are canceled. Thus, claims 1, 2, 26, and 27 are now before the examiner.

SEQ ID NO:7 is from *Bacillus thuringiensis* strain PS52A1.

Support for the amended claims can be found in paragraphs 52 (item 3.), 77-83, and in Example 5, for example. The Maniatis text is also cited in Example 7. Relevant pages of this text are enclosed.

Although it is believed that no fees are due in connection with this Election, the Commissioner is hereby authorized to charge any such fees to Deposit Account 19-0065.

The applicant invites the Examiner to call the undersigned if clarification is needed on any of this response, or if the Examiner believes a telephonic interview would expedite the prosecution of the subject application to completion.

Respectfully submitted,

Jay M. Sanders
Patent Attorney
Registration No. 39,355
Phone No.: 352-375-8100
Address: P.O. Box 142950
Gainesville, FL 32614-2950

JMS/ehm
Attachment: Maniatis *et al.*, *Molecular Cloning: A Laboratory Manual,* Cold Spring Harbor Laboratory (1982), pages 326-327

Molecular Cloning

A LABORATORY MANUAL

T. Maniatis Harvard University
E. F. Fritsch Michigan State University
J. Sambrook Cold Spring Harbor Laboratory

Cold Spring Harbor Laboratory
1982

HYBRIDIZATION TO NITROCELLULOSE FILTERS CONTAINING REPLICAS OF BACTERIOPHAGE PLAQUES OR BACTERIAL COLONIES

The following protocol is designed for (a) two 20-cm × 30-cm nitrocellulose filters or (b) 30 circular, 82-mm-diameter filters. Appropriate adjustments should be made to the volumes when carrying out hybridization reactions with different numbers or sizes of filters.

1. Float the baked filters on the surface of a tray of 6× SSC until they have become thoroughly wetted from beneath. Submerge the filters for 5 minutes.

2. Transfer the filters (a) to a rectangular, flat-bottomed plastic box (22 cm × 32 cm) or (b) to a circular, glass crystallizing dish. Stack the filters on top of one another.

3. Add (a) 300 ml or (b) 100 ml of prewashing solution. Incubate at 42°C for 1–2 hours.

   In this and all subsequent steps, the circular filters in the crystallizing dish should be agitated on a rotating platform so that they do not stick to one another. The large, rectangular filters may be stationary.

   The prewashing solution removes from the filters any absorbed medium, fragments of agarose, or loose bacterial debris.

   Prewashing solution

   50 mM Tris · Cl (pH 8.0)
   1 M NaCl
   1 mM EDTA
   0.1% SDS

4. Pour off the prewashing solution. Incubate the filters for 4–6 hours at 42°C in (a) 100–150 ml or (b) 60 ml of prehybridization solution.

   The filters should be completely covered by the prehybridization solution. During prehybridization, sites on the nitrocellulose filter that bind single- or double-stranded DNA nonspecifically become saturated by unlabeled, salmon sperm DNA, SDS, or components in the Denhardt's solution. When using ^{32}P-labeled cDNA or RNA as a probe, poly(A) should be included in the prehybridization solution and hybridization solutions at a concentration of 1 μg/ml to prevent the probe from binding to T-rich sequences that are found fairly commonly in eukaryotic DNA.

   Prehybridization solution

   50% formamide
   5× Denhardt's solution
   5× SSPE
   0.1% SDS
   100 μg/ml denatured, salmon sperm DNA

After all the components have dissolved, centrifuge the prehybridization solution at 1000*g* at 15°C for 15 minutes or filter it through Whatman 1MM paper using a Buchner funnel. Sterilize the solution by filtration through disposable Nalgene filters. Store frozen at −20°C in 25-ml aliquots.

Formamide. Many batches of reagent-grade formamide are sufficiently pure to be used without further treatment. However, if any yellow color is present, the formamide should be deionized by stirring on a magnetic stirrer with Dowex XG8 mixed-bed resin for 1 hour and filtering twice through Whatman 1MM paper. Deionized formamide should be stored in small aliquots under nitrogen at −70°C.

Denhardt's solution (50×)

Ficoll	5 g
polyvinylpyrrolidone	5 g
BSA (Pentax Fraction V)	5 g
H_2O	to 500 ml

20× SSPE. See page 314.

Denatured, salmon sperm DNA. This is prepared as follows: Dissolve the DNA (Sigma Type-III sodium salt) in water at a concentration of 10 mg/ml. If necessary, stir the solution on a magnetic stirrer for 2–4 hours at room temperature to help the DNA to dissolve. Shear the DNA by passing it several times through an 18-gauge hypodermic needle. Boil the DNA for 10 minutes and store at −20°C in small aliquots. Just before use, heat the DNA for 5 minutes in a boiling-water bath. Chill it quickly in ice water.

5. Denature the ^{32}P-labeled probe DNA by heating for 5 minutes to 100°C. Add the denatured probe to the prehybridization solution covering the filters. Incubate at 42°C until 1–3× $C_o t_{1/2}$ is achieved (see page 325). During the hybridization, the containers holding the filters should be tightly closed to prevent loss of fluid by evaporation.

6. After the hybridization is completed, discard the hybridization solution. Wash the filters 3–4 times, for 5–10 minutes each wash, in a large volume (300–500 ml) of 2× SSC and 0.1% SDS at room temperature. Invert the filters at least once during washing. At no stage during the washing procedure should the filters be allowed to dry.

7. Wash the filters twice for 1–1.5 hours in (a) 500 ml or (b) 300 ml of a solution of 1× SSC and 0.1% SDS at 68°C. At this point, the background is usually low enough to put the filters on film. If the background is still high or if the experiment demands washing at higher stringencies, immerse the filters for 60 minutes in (a) 500 ml or (b) 300 ml of a solution of 0.2× SSC and 0.1% SDS at 68°C.